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Urothelial Carcinogenesis and Portocaval Anastomosis in the Rat

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Summary. Portacaval anastomoses were performed in the rat to study urothelial carcinogenesis in this model and the promoting effect of dietary tryptophan. We were unable to produce any urothelial cancers or premalignant changes; some animals formed uric acid stones and developed papillary hyperplasia in the bladder. We conclude that the initiating carcinogen is likely to be exogenous and may be dietary in those experiments that have produced urothelial cancers.

Key words: Portacaval anastomosis, Urothelial carcinogenesis, Uric acid calculi, Dietary tryptophan.

Introduction

The portacaval anastomosis (PCA) in the rat has been used to study the pathophysiology of several different organs since a reliable microsurgical technique was developed by Lee and Fisher [9]. The first report that PCA produced changes in the urinary tract was made by Herz et al. [7] who showed that rats developed uric acid calculi and the defect in uric acid metabolism was investigated by Lauterburg et al. [8]. Heine et al. [6] first reported that all rats subjected to PCA developed hyperplastic and dysplastic changes progressing to invasive carcinoma in both upper urinary tracts and bladder. This has been repeated by other workers [2, 4, 5]. Grun et al. [5] showed that modifying the procedure to leave only the pancreatico-duodenal vein supplying portal blood directly to the liver prevented both hyperplastic and neoplastic changes developing in the urothelium. All the above studies used only male rats.

In our preliminary experiments [10] we showed that female rats did not develop uric acid calculi and neither male nor female rats developed urothelial carcinomas. This study was terminated at 40 weeks. Areas of papillary hyperplasia were found in the bladders of those male rats with calculi. This study was therefore repeated using male rats with added tryptophan to the diet to act as a tumour promoter as has been described following the induction of urothelial cancers in the rat by Acetylaminofluorine [3] and FANFT [1].

Materials and Methods

Male specific pathogen free Sprague-Dawley rats were bought from a commercial supplier (Süddeutsche Versuchstierfarm, Tuttlingen). The animals were operated on at a weight of 250–400 g using fentanyl-fluanisone anaesthesia supplemented by ether. A complete end-to-side anastomosis was performed using an operating microscope and the portal vein was divided above the pancreatico-duodenal vein which was also routinely divided. Eighty rats were operated on with an operative mortality of six (7.5%).

Fifty-four rats were then fed on a standard laboratory diet (Altromin 1324) and 20 rats were fed on the standard diet with 2% added DL-tryptophan. Thirty rats had a laparotomy only and were fed on standard diet and five rats had a laparotomy only and were fed on the added tryptophan diet. The animals were housed under standard conditions and allowed tap water ad libitum.

The animals were sacrificed at one year and the bladder was distended with 10% buffered formalin and excised. The bladder was transilluminated in order to detect the presence of stones or sand before being bisected and examined under a dissecting microscope. The bladders were then sectioned and stained for light microscopy.

Results

The survival of the animals with portacaval anastomoses is shown in Fig. 1. 88% of the animals with PCA alone died before one year whereas only 30% of the animals with the PCA who were fed on the tryptophan diet died within one year. All PCA animals showed a weight loss of at least 10% during the first month. None of the animals whose weight dropped to below 60% of the operative weight survived one year. The animals in the PCA alone group who survived one year had a mean body weight at sacrifice of 105% of the operative weight (range 76–145%) and the tryptophan

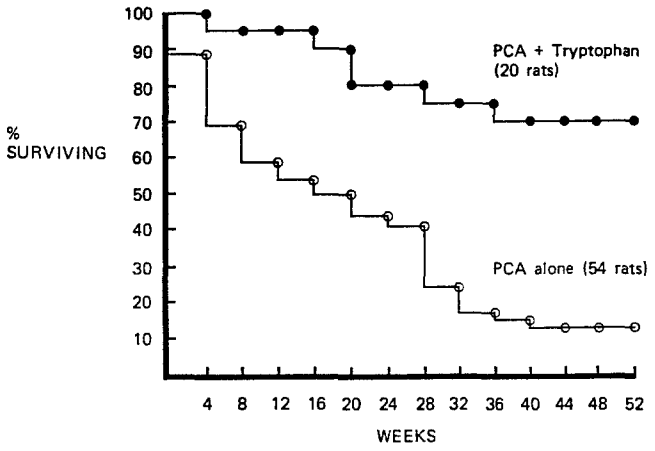

Fig. 1.

Table 1.

	Controls		PCA	
	Normal diet (n = 30)	Trypto-phan diet (n = 5)	Normal diet (n = 54)	Trypto-phan diet (n = 20)
Bladder stones and sand	2	2	5	11
Hyperplasia	0	0	1	3

group had a body weight of 102% (range 77–123%). All the control animals survived with a mean weight gain of 180% (range 145–278%).

At post-mortem examination all the portacaval anastomoses were patent and there was no evidence of a collateral circulation developing to the porta hepatis. There was no evidence of tumours or stones in the upper tracts of the PCA animals. In the PCA alone group 4 animals had bladder stones and 4 had sand in the bladder. 4 of the controls had stones in the bladder and none had sand. Chemical analysis of the stones showed that they were ammonium hydrogen urate.

One PCA alone animal and 3 PCA tryptophan diet animals had tumours visible in the bladder under the dissecting microscope. All these tumours occurred in association with bladder stones and on microscopy were papillary hyperplasias with no evidence of any dysplasia or invasion. Inflammatory changes were also present in the submucosa. No tumours were seen in any of the control animals (Table 1).

Discussion

Uric acid lithiasis and urothelial carcinogenesis were both incidental findings during long term experiments with the PCA in the rat and were not reported until 11 and 18 years after the model was first described. The mechanism of urothelial carcinogenesis in this model may be either the failure to inactivate carcinogens in the diet or the endogenous production of urothelial carcinogens as a result of the widely disordered metabolism in the rat following PCA. It is important that the mechanism of urothelial carcinogenesis in this model is understood as the rat is widely used to study experimental urothelial carcinogenesis and for testing various therapeutic modalities. Portacaval shunting procedures are also performed on children and young adults with good long term survival though there are not as yet any reports in the literature of urothelial cancers occurring in such patients.

In our experiments we were unable to reproduce the results of Heine et al. [6], Duy et al. [4] or Dubuisson et al. [2]. The striking difference is that those workers who have produced tumours have reported that tumours occur in all animals that have survived for one year and pre-malignant changes are apparent after only four months. We have not found any pre-malignant changes after one year and the papillary hyperplasias that we saw were thought to be reactive in association with bladder calculi. The operative techniques appear to have been exactly the same and the histological effects on the liver were similar to those seen by Heine (personal communication). All groups have also observed a high incidence of uric acid calculi. The strain of rats used has been different; the diets used by Heine et al. [6] and Dubuisson et al. [2] were of Altromin but its composition may have varied as the experiments were carried out at different times and places. It therefore seems likely that an exogenous initiating carcinogen which was present in some experimental systems but not in ours could account for the finding of urothelial cancers in nearly all animals in the above experiments and no evidence of any urothelial cancers in our study.

Grun et al. [5] showed that preneoplastic and neoplastic changes could be prevented by a modified shunt which left the gastroduodenal vein intact supplying some blood to the liver. Renal bile acid excretion was the same in both groups and it was concluded that bile acid excretion was not related to the development of urothelial cancers. None of the animals in our series had the gastroduodenal vein left supplying blood to the liver.

The addition of tryptophan to the diet did not have any detectable initiating or promoting effect. The markedly improved survival in this group has not been explained and may merit further study.

The PCA in the rat is therefore an unreliable method of producing urothelial cancers which results in a high morbidity and mortality. Further study can only be recommended for those centres that have already been able to produce urothelial cancers and to study in particular the role of exogenous dietary carcinogens.

References

1. Cohen SM, Jacobs JB, Friedell GH (1979) Promoting effects of saccharine and DL tryptophan in urinary bladder carcinogenesis. Cancer Res 39:1207–1217
2. Dubuisson L, Vonnahme F-J, Balabaud Ch, Grun M (1984) Neoplastic surface changes in urothelium of rats after portacaval anastomosis. A combined light and scanning electron microsciopic study. Exp Pathol 26:49–58
3. Dunning WF, Curtis MR, Maun ME (1950) The effect of added dietary tryptophan on the occurrence of 2-acetylaminofluorine-induced liver and bladder cancer in rats. Cancer Res 10:454–459
4. Duy N, Yagamuchi Y, Prabhudesai M, Babb J, Gans H (1981) Cancer of the bladder in the portacaval shunted rat. Gastroenterology 80:1331
5. Grun M, Richter E, Heine WD (1982) Renal bile acid excretion as a cause of neoplastic lesions in the urinary tract after total portal shunt in the normal rat? Hepatogastroenterology 29:232–235
6. Heine WD, Grun M, Rasenack U, Leihr H (1979) Präneoplastische und neoplastische Urothelveränderungen nach portacavaler Anastomose der Ratte. Histologische und autoradiologische Befunde. Verh Dtsch Ges Pathol 63:517
7. Herz R, Sautter V, Bircher J (1972) Fortuitous discovery of urate nephrolithiasis in rats subjected to portacaval anastomosis. Experientia 28:27–28
8. Lauterburg B, Sautter V, Herz R, Colombo JP, Roch-Ramel F, Bircher J (1977) The defect of uric acid metabolism in Eck-fistula rats. J Lab Clin Med 90:92–100
9. Lee SH, Fisher B (1961) Portacaval shunt in the rat. Surgery 50:668–672
10. Wallace DMA, Ackermann D, Davis B, Hartmann WH (1985) Uric acid lithiasis and proliferative changes in the rat urinary bladder after portacaval anastomosis. In: Harzmann R, Jacobi GH, Weissbach L (eds) Experimentelle Urologie. Springer, Berlin, pp 430–433

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